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U1S S1289 S2419

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EP 0261940 A EP 0259212 A WO 88/02022 A
Annual Report (1987) of Institute For Animal Health "Capripox virus as a vector"

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(54) Recombinant capripoxvirus

(57) A heterologous gene encoding an antigen of a pathogen, for example an antigen associated with a viral or other disease of sheep, goats, cattle etc., is inserted in a non-essential region of the capripoxvirus genome, for example the TK (thymidine kinase) coding region or the open reading frames designated herein as CT3C, CT4 or Q2. The recombinant virus may be used to make vaccines, in which the virus is preferably in an attenuated form rather than killed, to prevent the disease (5) associated with the pathogen.

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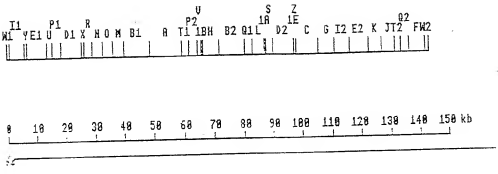
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P1
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B1
A
U
P2
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B2 Q1
S
1A
L
Z
1E
D2
C
G I2 E2 K
Q2
JT2
FW2
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 kb

Figure 1

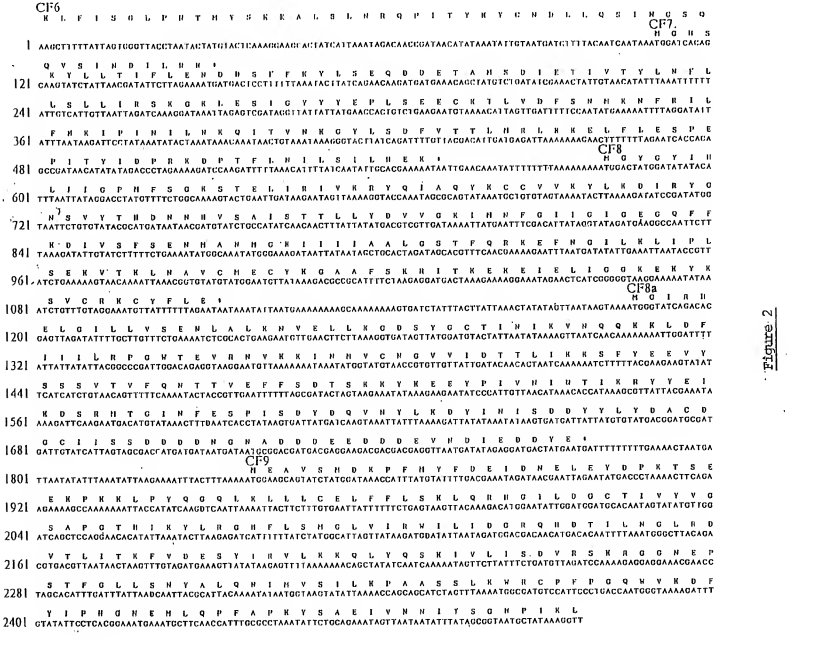

Figure 2

GATCATTTCCAAATACAAGTGAGGCATCCTTTTTGAAAGATTCAAAAACTA
AGAACACCTTTCCAGCAACCTCCCTGGACGAAAATGCCAGTGATGACCAA
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CCTTTATTGTGTTTTGATTACAGTAGTATTCGTAGTGTTTTTTAAATGGA
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ACAAATTCTAAATCATAATCTTTAAAACTTACACTCTCAACGTATGATTT
CATTTCTGTAGTTACTTGATCACAAAGAAATTGAC-ATGATATTCTCTTA
GCACACTGTGGCAGCGAATACAGAGTGTTATATTACTGGTTGATATTTCC
ATATATACATATTTACCCAATGCATAAACTGCTTTGTTAACACTATCTAA
TATAAAGTTGTTTTGTGTTTCTTCTTTGGAACAATCATCATGTGCGAAAA
TTTTAAATCGATTTCCCTCTGTTAGAAGTCCTCCAGGTCCTTGAATTTTA
TAACAGCCAGTTTTAATCGCTCTAAAATTTTCTGTTTGGATACATAATCC
TACTTCGATTTCCCATGTGTTATTTTCTTCTTC-GTGCATCTCTTTATTG
ATATT-CGCTTACACTACTAGTAAAAATGAATAGTTGCAAAAAGAAAATG
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Figure 3

Sequence length = 1649 nt
Full scale = 1649 nt
Left end inset = 0 nt

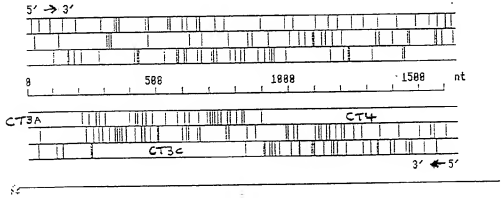

Figure 4

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VACCINE

The present invention relates to vaccines in which a viral vector is used to express a heterologous gene.

Capripoxvirus causes sheep pox and goat pox, diseases which can kill over 50% of young animals in those areas, principally Africa and the Middle and Far East, where the disease is enzootic. Different strains of the virus show a preference for either sheep or goats but none have so far been found to be totally species-specific. Some strains also infect, and cause lumpy skin disease in, cattle, but (apart from buffalo) capripoxviruses do not infect any other domestic animals. A vaccine has been developed which comprises an attenuated strain of the strain known as "the Kenya isolate" (Kitching, Rev. Sci. Tech. Off. Int. Epiz., 1986, 5(2), 503-511).

It is known that the vaccinia virus genome can be altered by insertion of a heterologous gene from a pathogen, such that the product of that gene will be expressed in infected cells.

Protection against diseases associated with the pathogen has been induced in this way. A review of such work may be found in Mackett & Smith, J. Gen. Virol. 67. 2067-2082 (1986).

At a U.N. Food and Agriculture Organisation meeting in Madrid in October 1986, Mowat disclosed that capripoxvirus represented a possible alternative to vaccinia as a vector for foot and mouth disease vaccines. In the 1987 Annual Report of the Institute for Animal Health, Compton, U.K., (published April 1988) Black and Gershon disclosed that work was in progress on the use of capripoxvirus as a vector for heterologous antigens and that the thymidine kinase gene was a candidate insertion site.

We have now shown that capripoxvirus can indeed be used as a vector for heterologous genes and that there are several possible insertion sites for such genes.

One aspect of the invention provides a capripoxvirus capable of infecting and replicating in capripoxvirus-susceptible cells and carrying at least one heterologous gene

encoding an antigen of a pathogen such that the gene will be expressed during transcription and translation from the capripoxvirus genome in the said cell.

Preferably, the heterologous gene is inserted in one of the following regions of the capripoxvirus genome:

(a) the thymidine kinase gene region,

(b) the CT3C region,

(c) the C4 region, and

(d) the Q2 region

By "thymidine kinase gene region" we mean the genetic unit, including coding and non-coding sequences, for the expression of thymidine kinase.

By "CT3C region" and "CT4 region", we mean the respective regions of the capripox virus genome identified by these designations in the attached figures, or variations thereof. By "Q2 region", we mean the approximately 2.4 kb fragment bounded by the respective HindIII sites approximately 6.6 and 9.0 kb from the right-hand end of the KS-1 isolate genome shown in Figure 1 and identified thereon as Q2, and variations

thereof. Such variations of any of these regions will include: minor polymorphic changes associated with different strains of capripoxvirus; any other changes which might occur or be created in the nucleic acid of a capripoxvirus but which do not alter the non-essential nature of the said region or the nature of the gene product encoded by that region; and any other nucleotide sequences which encode a gene product with substantially the same structure or function as the gene product of the said region or which correspond to the said region but are non-coding. In general, nucleotide sequences with at least 80% homology to the said regions are regarded as variations thereof. Preferably, the extent of homology is 85%, 90%, 95% or 99%.

By "antigen" we mean any structure, part thereof or mimic thereof associated with a pathogen and capable of giving rise to an immune response in a host when the immune system of that host is exposed to the antigen. Preferably, the immune response is a protective response. Thus, surface carbohydrates, glycoproteins and proteins of viruses and other pathogens are included in this definition, as are peptides which are homologous to part of such whole molecules, and peptides which mimic the tertiary structure of such molecules.

In the case of antigens which are glycoproteins, the heterologous gene will encode the amino acid sequence of the glycoprotein and/or one of more enzymes which cause appropriate glycosylation of that amino acid sequence. In the case of antigens which are carbohydrates, the heterologous gene or genes will encode one or more enzymes which catalyse the synthesis of such carbohydrates.

By selecting appropriate heterologous genes from other pathogens, it is possible to use the vectors of the invention in vaccines effective in preventing a wide variety of diseases in sheep, goats and cattle. The following is a non-exhaustive list of such diseases:

Viral disease	Host
Rotavirus infection	Sheep, cattle
* Bluetongue	Sheep, goats, cattle
+ Ephemeral fever	Cattle
* Foot-and-mouth disease	Sheep, cattle, goats, wild ruminants
Infectious bovine rhinotracheitis	Cattle

Infectious pustular vulvovaginitis	Cattle
Malignant catarrh	Sheep, cattle
+ Mucosal disease/bovine viral diarrhoea	Cattle
+ Border disease	Sheep
+ Rabies	Sheep, cattle, goats, wild ruminants
Rift Valley fever	Sheep, cattle, goats, wild ruminants
* Rinderpest	Cattle, goats
* Peste des petits ruminants	Sheep, goats
+ Vesicular stomatitis	Sheep, goats, cattle
Nairobi Sheep disease	Sheep
Scrapie	Sheep
+ Lentivirus infection	Sheep, goats
Akabane	Cattle
Ricketsial disease	Host
Anaplasmosis	Cattle
Bovine petechial fever	Cattle
Heartwater	Cattle
Jembrana disease	Cattle
Enzootic abortion	Sheep
Bacterial disease	Host

Anthrax	Sheep, goats, cattle, wild ruminants, buffalo
Brucella abortus infection	Buffalo, cattle
Brucella melitensis infection	Cattle, sheep, goats, buffalo
Brucella ovis infection	Sheep
Clostridium septicum infection - blackleg) malignant oedema) braxy)	Sheep, cattle, goats, buffalo
Clostridium chauvoei infection - blackquarter	Sheep, cattle, goats, buffalo
Clostridium novyi (type A) infection - Big head	Sheep
Clostridium perfringens infection	Sheep, cattle, goats, buffalo
Clostridium novyi (type B) infection - Black disease	Sheep, cattle, goats
Clostridium novyi (type D) infection - Bacillary Haemoglobinuria	Cattle, sheep, goats

Clostridium perfringens (type B) infection - Lamb dysentery	Sheep
Clostridium perfringens (type C) infection	Sheep, cattle
Clostridium perfringens (type D) infection - Pulpy kidney disease	Sheep, goats
Clostridium tetani infection - Tetanus	Sheep, goats, cattle, wild ruminants
Clostridium botulinum infection - Botulism	Sheep, goats, cattle, wild ruminants
Mycoplasma agalactia infection - Contagious agalactin	Sheep, goats
Mycoplasma mycoides infection - Contagious bovine pleuro-pneumonia	Buffalo, cattle, wild ruminants
Contagious caprine pleuro-pneumonia	Sheep, goats
Pasteurella multocida infection - Haemorrhagic septicaemia	Cattle, buffalo
Mycobacterium johnei infection - Johne's disease	Cattle, sheep, goats,

	buffalo
Leptospira sps. infections	Cattle, sheep, goats, buffalo
Salmonella sps. infections	Cattle, sheep, goats, buffalo
Mycobacterium tuberculosis infection	Cattle, sheep, goats, buffalo
Pasteurella sps. infection	Sheep, goats

Protozoal disease	Host
Babesia sps. infections	Cattle, buffalo, sheep, goats, wild ruminants
+ Theileria sps. infections	Cattle, buffalo, sheep, goats, wild ruminants
+ Toxoplasma gondii infection	Sheep
+ Trypanosoma sps. infections	Cattle, buffalo, sheep, goats, wild ruminants

Helminth disease	Host
Bunostomum sps infection	Sheep, goats, cattle, buffalo, wild ruminants
Chabertia sps. infection	Sheep, goats, cattle, buffalo, wild ruminants

Taenia sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Trichuris sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Dicrocoelium infection	Sheep, goats, cattle, buffalo, wild ruminant
Dictyocaulus sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Echinococcus sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Fasciola sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Haemonchus sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Oesophagostomum sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Onchocerca sps. infection	Cattle, buffalo
Ostertagia sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Paramphistoma sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Schistosoma sps. infection	Sheep, goats, cattle buffalo, wild ruminants

Stephanofilaria sps. infection	Cattle, buffalo
Strongylus sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Thelazia sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Trichostrongylus sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Cooperia sps. infection	Sheep, goats, cattle, buffalo, wild ruminants
Nematodirus sps. infection	Sheep, goats, cattle, buffalo, wild ruminants

Genes have already been isolated for antigens in those viruses marked with an asterisk or a cross.

The recombinant viruses of the invention are prepared by (i) constructing a DNA construct comprising the heterologous gene and two sequences which flank the heterologous gene and which are homologous to respective parts of the capripoxvirus DNA, such that recombination of the construct with capripoxvirus may occur, (ii) infecting cells with capripoxvirus and introducing the said construct into the said

cells, (iii) screening the infected cells for recombinant capripoxvirus, (iv) isolating the recombinants from step (iii) and (v) growing the isolated viruses in a suitable host.

The construct may comprise a promoter for the heterologous gene, which will be any promoter which is functional in the cytoplasm of the capripoxvirus-infected cells. Thus, the promoter may interact with a polymerase which is also heterologous to both the capripoxvirus and the infected cell and which is encoded by another heterologous gene which is inserted in the recombinant capripoxvirus, as with the bacterial T7 system. Suitably, the promoter is derived from capripoxvirus itself but it may alternatively be derived from vaccinia virus, for example it may be the promoter for the P7.5 protein (Mackett et al (1984), J. Virol. 49, 857-864; and Mackett & Smith, loc. cit.). Alternatively, the construct may not comprise a promoter at all but is so constructed as to recombine with the capripoxvirus at a site close to, and in correct reading frame with, an endogenous capripoxvirus promoter.

The flanking sequences may be synthesised by reference to the sequence of the insertion region or may be obtained from such a region and cloned. Typically, a length of about 100 nucleotides for each flanking sequence is sufficient to ensure recombination, but 120 nucleotides or more may be used.

In step (ii), the infection of the cells with the capripoxvirus may be before, after or at the same time as introduction of the DNA construct. The cells may be any which are permissive for capripoxvirus, such as most ovine, bovine and caprine derived cells, for example lamb testis, bovine palate, or ovine kidney cells. The DNA construct is preferably provided as a plasmid. The plasmid may be based on commercially available bacterial plasmids, such as pUC9, which may be cloned in bacteria and which have suitable markers for selection, for example Amp^r. Transfection of the capripoxvirus-susceptible cells with the plasmid may be facilitated by known techniques involving calcium phosphate or electroporosis.

The plasmid or other transfection vehicle is so chosen as to be non-infectious. Following introduction of the transfection vehicle into the host cell in step (ii), the

vehicle is not propagated between cells. Hence, screening in step (iii) for the product of the heterologous gene effectively screens for recombinant viruses. Such screening may, for example, comprise an immunological assay for the antigen expressed by that gene or nucleotide hybridisation using sequences homologous to the heterologous gene. In one screening approach, the heterologous gene is in the first instance the E.coli beta-galactosidase gene (lac Z), thus allowing screening in step (iii) by the well-known technique using "X-gal" (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) in an agar overlay on the cell culture. Blue plaques are formed by recombinants which express the lacZ gene. Insertion of a further heterologous gene into the recombinant lac Z region, with concomitant deletion of the beta-galactosidase function, then allows selection by isolating colourless plaques against the parental blue plaques. This approach may be useful where a suitable assay for the product of the second heterologous gene is not available.

Step (iv) will usually involve successive rounds of purification from plaques or cell-foci of infection, or end-point dilutions, and screening for recombinants in known ways. Amplification may be desirable during such procedures.

In step (v) the host may be any suitable cell line or, less preferably, any suitable living animal. Lamb testis and the other cell lines mentioned above are suitable.

The recombinant viruses may then be used to make vaccines in known ways, for example as described in Kitching, loc.cit. The virus is preferably included in the vaccine in an attenuated form, rather than killed.

The vaccines may be used to prevent the particular disease or diseases associated with the pathogen(s) of which an antigen or antigens are encoded by the virus of the invention, and may be used in sheep, goats, cattle (Bos bovis and Bos indicus), buffalo, llamas, alpacas and vicunas. Generally speaking, the vaccines will be most suitable for use in areas where capripoxvirus-associated diseases are endemic and/or where the animals have all been vaccinated against capripoxvirus.

The vaccines may be used to prevent the particular disease or diseases associated with the pathogen(s) of which an antigen or antigens are encoded by the virus of the invention, and may be used in sheep, goats, cattle (Bos Bovis and Bos

indicus), buffalo, llamas, alpacas and vicunas. Eventually speaking, the vaccines will be most suitable for use in areas where capripoxvirus-associated diseases are endemic and/or where the animals have all be vaccinated against capripoxvirus.

The vaccines may be administered by any suitable route, including intra-nasally (as a spray), orally (for example in the feed or drinking water), intra-muscularly, sub-cutaneously, intra-venously or intra-dermally. However, experience with existing capripoxvirus vaccines shows that the intra-dermal route is not preferred and that the sub-cutaneous or intra-muscular routes are preferred.

For at least some time, typically 10 days, after the vaccine is administered to the animal, especially when the virus is attenuated rather than killed, the virus will be detectable in the animal's tissues, thus enabling an animal which has been vaccinated in accordance with the invention to be identified. The viral vectors of the invention may further include markers to aid such identification, if desired. In addition, the immunity of an animal which has been vaccinated in accordance with the invention may be distinguished from that of an animal which has been exposed to the corresponding

pathogen since the former will include antibodies only to the particular antigen expressed by the modified viral genome and not to other antigens of the pathogen.

The invention will now be illustrated by way of the following non-limiting examples, with reference to the accompanying drawings, in which:-

Figure 1 shows the distribution of HindIII restriction enzyme recognition sites on the genome of capripoxvirus strain KS-1, together with a scale representing length in numbers of kilobase pairs;

Figure 2 shows the nucleotide sequence of the S fragment identified in Figure 1, with the region labelled as CF8 corresponding to the thymidine kinase (TK) region;

Figure 3 shows the sequence from the terminal region of the genome of capripoxvirus isolate KS-1, reading in a telomer-proximal to telomere-distal direction. The terminal HindIII site (AAGCTT) of the genome occurs in the sequence. This site, and the Sau3A1 (GATC) site defining the proximal end of clone pPG64, are underlined and emboldened; and

Figure 4 shows the distribution of stop-codons in the 6 possible reading frames for the sequence between approximately 700 and 2,400 bp from the apex of the terminal hairpin loops of capripoxvirus strain KS-1 DNA.

EXAMPLE 1 : Insertion into the TK region

The thymidine kinase (TK) gene of capripoxvirus was localized on the genome of isolate KS-1 (previously referred to as 'Kenya Sheep and Goat Pox') as described in reference 1. The gene was found to be present within HindIII fragment S (as shown on Fig. 1), and HindIII S was completely sequenced.

To insert foreign DNA into the TK gene of capripoxvirus, we used a variation of the previously described method (Mackett & Smith, loc. cit.) of: cloning a construct comprising (i) the genomic region containing the candidate insertion site in a bacterial plasmid and (ii) a foreign gene attached to a poxvirus promoter at the site in the plasmid clone equivalent to the intended insertion site in the virus genome; and transfecting the recombinant plasmid into poxvirus infected cells.

In the experiments performed here, the genomic region containing the intended insertion site was the KS-1 genomic DNA fragment HindIII S, which was cloned in the plasmid vector pUC9. The plasmid was linearized by incubating with the restriction enzyme KpnI, for which a single site is present in the clone, and is located within the KS-1 TK gene.

The bacterial lacZ gene coupled to the vaccinia virus promoter P7.5 (P7.5-lacZ), was inserted at the KpnI site in the above plasmid using T4 ligase, after cleaving P7.5-lacZ from another plasmid with the restriction enzyme BamHI, and treating both the BamHI and the KpnI fragments with Klenow enzyme. The resulting plasmid was named pPG55.

Lamb testis (LT) cell cultures infected with capripoxvirus isolate KS-1 were transfected with the pPG55, and the cultures were incubated at 37°C for 5 days. Further LT cell cultures were infected with the harvested virus and after seven days incubation these cultures were overlaid with low gelling temperature (LGT) agarose containing the substrate X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) ('X-gal overlay'). After a further 24 hours blue foci were

visualised through the X-gal overlay, virus was picked through the agarose and subjected to further rounds of plaque purification.

EXAMPLE 2 : Identification of Further Insertional Sites

A library of capripoxvirus KS-1 DNA in plasmid pPG2 was produced by partial digestion of the capripoxvirus DNA with Sau 3A, and cloning of the resulting fragments into BamH1 linearised pPG2. pPG2 was derived from the commercially available pUC9 by removal of the multiple cloning site by digestion with HindIII and EcoR1, filling in of the single-stranded ends with Klenow enzyme, and ligation (using T4 ligase) of a synthetic BamHI linker (GGGATCCC) to recircularise the plasmid DNA. The ligation mixture was used to transform E.coli strain TG1 and the bacteria plated onto LB agar containing ampicillin. Individual bacterial colonies were picked at random and their plasmid DNAs prepared. Plasmid DNAs containing HindIII sites were identified by digestion with HindIII and analysis by agarose gel electrophoresis. 40 clones carrying HindIII sites located at different positions on the capripoxvirus genome were digested with HindIII and the HindIII P7.5-lacZ fragment was inserted into each using T4 ligase to

create a series of trial insertional vectors for at least 10 different sites in the capripoxvirus genome. Following cloning in E.coli and preparation of purified plasmid DNA, each trial insertional vector was transfected into capripoxvirus KS-1 infected lamb testis cells and the presence of blue plaques/foci was screened for. The presence of blue plaques/foci identified those insertional vectors carrying sequences which directed insertion by recombination at sites which are not essential for capripoxvirus replication and infection in lamb testis cells. In this way we have identified three HindIII sites within non-essential regions of capripoxvirus DNA into which heterologous DNA can be inserted.

This represents a general method for identifying non-essential insertion sites in the capripoxvirus genome. Other restriction enzymes may be used instead of HindIII, preferably those which (for the capripoxvirus genome) cut at about the same frequency, namely about 25-35 times, preferably about 30 times. Infrequent cutting results in insertion sites being missed, whereas too frequent cutting yields fragments which are too small to be practicable. Generally, one would use enzymes which recognise a sequence of 5 to 7 nucleotides, preferably 6.

However, as the capripoxvirus genome is rich in A's and T's, an enzyme recognising a 4-nucleotide sequence rich in G's or C's might also be suitable.

Sau3A is a suitable enzyme for the initial digestion as it recognises frequently occurring sites (about 600, i.e. an average distribution of about one per 250 b.p.); a partial, rather than complete, digest then yields virtually random, conveniently-sized fragments likely to contain a HindIII site. Other enzymes, such as HaeIII, may be used for differing frequencies of occurrence of the recognition sites. The partial digests may be pooled. The pool itself may be cloned or one may select a particular size class of fragment for cloning.

EXAMPLE 3 : Insertion into the CT4 Region

The work outlined in this Example is based on clones covering a region within the terminal repeats of the genome of capripoxvirus isolate KS-1. This region covers two open reading frames (ORFs) named CT3C and CT4. Figure 3 shows the 1651 b.p. nucleotide sequence of the above mentioned region. Figure 4 shows the positions of the stop codons in the

sequence. ORFs CT3C and CT4 are marked; CT3C lies between nucleotide 252 and 647 and CT4 lies between nucleotide 839 and 1621 in this sequence.

We constructed a plasmid, pPG64, to enable P7.5-lacZ to be inserted within CT3C; more specifically at the Hind III site between nucleotides 561 and 566 of the sequence shown in figure 3. This HindIII site divides HindIII fragments W1 and 11 at the left-hand end of the genome and fragments F and W2 at the right-hand end (see figure 1). Clone pPG64 was made by ligating a Sau3A partial digest fragment of KS-1 DNA of size 1648 b.p., starting at nucleotide 440 on the sequence shown in figure 3, in a derivative of the commercially available plasmid cloning vehicle pUC9, named pPG2 (see Example 2). The resulting plasmid, in which P7.5-lac2 is cloned at the HindIII site of pPG64, is called pPG65.

Recombinant capripoxvirus KS-1 was generated using pPG65 by the method described in the last paragraph of Example 1. In the experiments using pPG65, virus obtained from blue viral foci was propagated on LT cells, and gave a further round of

blue foci. These in turn were propagated by several additional rounds of plaque purification and shown to maintain the ability to express blue foci.

The capripoxvirus isolates used in the work described herein are typical of wild-type capripoxvirus and the techniques described and claimed herein do not depend upon the particular strain or isolate which is used. Other isolates may have minor variations in their genomes, but it will be well within the capabilities of the man skilled in the act to adapt the techniques to such other isolates. For example, it is believed that the region of another isolate (InS; Gershon & Black (1987) Virol. 160, 473-476) which corresponds to the ORF in KS-1 labelled herein as CT4 is a non-coding region. Such a region would clearly be a suitable candidate for insertion of the heterologous gene.

CLAIMS

1. A capripoxvirus capable of infecting and replicating in capripoxvirus-susceptible cells and carrying at least one heterologous gene encoding an antigen of a pathogen such that the gene will be expressed during transcription and translation from the capripoxvirus genome in the said cell.

2. A capripoxvirus according to Claim 1 wherein the heterologous gene is inserted in one of the following regions of the capripoxvirus genome:

(a) the thymidine kinase region
(b) the CT3C region,
(c) the CT4 region, or
(d) the QZ region

the regions being as defined herein.

3. A capripoxvirus according to to Claim 1 or Claim 2 wherein the antigen is associated with bluetongue disease, ephemeral fever, foot-and-mouth disease, mucosal disease, border disease, rabies, Rift Valley fever, rinderpest, peste des petits ruminants, vesicular stomatitis, Nairobi Sheep disease, lentivirus infections, trypanosomiasis, theileriosis, clostridial infections, tuberculosis, haemonchiosis, schistosomiasis or Brucella infections.

4. A vaccine comprising a capripoxvirus according to any one of the preceding Claims and a carrier and/or adjuvant.

5. A vaccine according to Claim 4 wherein the vaccine is suitable for sub-cutaneous delivery.

6. A process for preparing a capripoxvirus according to any one of Claims 1 to 3 by (i) constructing a DNA construct comprising the heterologous gene and two sequences which flank the heterologous gene and which are homologous to respective parts of the capripoxvirus DNA such that recombination of the construct with capripoxvirus may occur and (ii) infecting cells with capripoxvirus and introducing the said construct into the

said cells, (iii) screening the infected cells for recombinant capripoxvirus, (iv) isolating the recombinants from step (iii) and (v) growing the isolated viruses in a suitable host.

7. A process according to Claim 6 wherein the construct comprises a promoter for the heterologous gene, the promoter being derived from capripoxvirus.

8. A method of treating or preventing pathogen-caused disease in cattle, buffalo, sheep or goats comprising administering to the cattle, buffalo, sheep or goats a non-toxic disease-protective amount of a vaccine according to Claim 4 or 5.

9. A cow, buffalo, sheep or goat treated by the method of Claim 8.

10. A method of identifying non-essential insertion sites in a capripoxvirus genome, comprising (i) preparing a library of capripoxvirus DNA in a suitable plasmid or other vector, (ii) transforming a culture of a suitable host with at least part of the said library, (iii) plating out the transformed host cells to give individual colonies, (iv) isolating plasmid DNA from

the said individual colonies, (v) digesting the said DNA with a suitable restriction enzyme, (vi) forming a construct by inserting into the insertion site thus formed a heterologous gene encoding a marker protein, (vii) transfecting host cells with the said construct, and (viii) screening for host cells which (a) are infected with live poxvirus and (b) express the marker protein.

11. A method according to Claim 10 wherein step (i) includes partial digestion with Sau3A.

12. A method according to claim 10 or 11 wherein the restriction enzyme of step (v) recognises 25-35 sites on the poxvirus genome.

13. A method according to Claim 12 wherein the restriction enzyme of step (v) is HindIII.

14. A method according to any one of Claims 10 to 13 wherein the marker protein is beta-galactosidase.

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